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A major role of PKC θ and NFκB in the regulation of hTERT in human T lymphocytes

Wei-Yun Sheng, Yi-Ru Chen, Tzu-Chien V. Wang*

Department of Molecular and Cellular Biology, Chang Gung University, Kwei-San ,Tao-Yuan 333, Taiwan

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Abstract Expression of the telomerase catalytic subunit (TERT) is the rate-limiting determinant of telomerase activity in most human cells. In this work, we examined the participation of protein kinase C (PKC) in the regulation of hTERT expression in human T lymphocytes. Transient expression assays using luciferase reporter plasmids containing hTERT promoter showed that overexpression of PKC θ, but not the other PKC isoforms, could activate the promoter activity of hTERT in resting T lymphocytes. Among the PKC θ-activated signalings, we presented evidence that the expression of hTERT is mediated through NFκB but not through MEK or c-Jun N-terminal kinase pathways. Analysis of the hTERT promoter occupancy in vivo using chromatin immunoprecipitation assays, however, did not detect an increased binding of NFκB to the hTERT promoter in the activated T cells, although an increased binding of cMyc and Sp1 was detected. Together with the observation that inhibition of NFκB eliminated the induction of cMyc in activated T cells, these results suggest that PKC θ-activated NFκB signaling regulates the expression of hTERT via cMyc in human T lymphocytes.

Keywords: NFκB; hTERT; Telomerase; T lymphocytes; cMyc; Phytohemagglutinin

1. Introduction

Human telomerase is a ribonucleoprotein complex composed of an RNA component (hTER), a reverse transcriptase protein subunit (hTERT) and several associated proteins [1]. Telomerase directs the synthesis of telomeric repeats at chromosome ends and is known to play a key role in maintaining telomere length and in replicative senescence [2]. It has been widely accepted that telomerase activation is crucial for transformed cells to stabilize their telomere length and to acquire infinite replicative potentials during oncogenic process, whereas most normal somatic cells undergo telomeric attrition and replicative senescence because telomerase is stringently repressed [3,4]. However, substantial levels of telomerase activity are seen in some normal tissues, such as male germ cells, activated lymphocytes and in certain types of stem cell populations [5,6]. The regulated expression of telomerase in highly proliferating normal cells suggests a functional role of telomerase in these cells [7,8]. So far, such tightly proliferation-regulated telomerase expression in normal cells has been poorly understood.

The regulation of telomerase activity is known to occur at various levels, including transcription, mRNA splicing, maturation and modification of hTER and hTERT, transport and subcellular localization of each component, and assembly of active telomerase [1]. Among the several components of human telomerase, only the catalytic component hTERT seems to be the rate-limiting determinant of telomerase activity. For examples, studies on the correlation between telomerase activity and the expression of hTER, hTEP1, and hTERT have indicated that telomerase activity is strongly correlated with the abundance of hTERT mRNA [9,10], but not the abundance of mRNA for hTEP1 and hTER [11–13]. Similarly, ectopic expression of hTERT in several somatic cells has been shown to be sufficient to restore telomerase activity [14–17], indicating that hTERT is the key regulator of enzyme activity. Expression of hTERT in human cells is regulated primarily at the level of transcription [1]. Sequence analysis has revealed that the hTERT promoter contains hormone response elements and binding sites for several transcription factors including cMyc, Spl, NFκB and others [18–20], implicating that *hTERT* expression may be regulated by multiple factors. Several activators (cMyc, Spl) and repressors (WTI, Mad1, E2FI, p53, Menin, MZF-2, etc.) for hTERT expression have been identified [1,21–23]. Exactly how these activators and repressors function in the different types of cells to regulate the expression of hTERT remains largely unknown.

T lymphocytes, a key player in adaptive immunity, require extensive cell division and clonal expansion for their functions. To achieve this, T lymphocytes may develop regulatory mechanism to overcome the problem of replicative senescence. It has been reported that the expression of telomerase activity is stringently regulated during T lymphocyte development and differentiation [6]. Telomerase activity is low or undetectable in peripheral T lymphocytes, but is upregulated several hundredfold upon activation [6,24]. Activation of T cells through T cell receptor is known to mediate through complex signaling pathways including the activation of protein kinase C (PKC)-dependent signaling pathways [25–27]. Indeed, the expression of telomerase activity in T lymphocytes has been shown to require PKC-dependent activities [28–30]. As yet, the molecular details for the participation of PKC-dependent signaling in the activation of telomerase remain largely unknown. In this work, the specific PKC isoform involved and the components of PKC-dependent signaling were investigated for their participation in the induction of hTERT expression in T lymphocytes.

*Corresponding author. Fax: +886 3 211 8700.
E-mail address: tcvwg@mail.cgu.edu.tw (T.-C.V. Wang).

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2. Materials and methods

2.1. Chemicals, enzymes, antibodies, and oligonucleotides

RPMI medium 1640, Hanks' balanced salt solution (HBSS), Superscript II reverse transcriptase, TRIzol reagent, antibiotics, and phytohemagglutinin (PHA) were purchased from Sigma Co., RNase inhibitor, MAP kinase kinase (MEK) inhibitors 2′-amino-3′-methoxyflavone (PD98059) and bis[amino[(2-aminophenyl)thio]methylene] butanedinitrile (U0126), c-Jun N-terminal kinase (JNK) inhibitor anthra[1,9-cd]pyrazol-6(2H)-one (SP600125), and NFκB inhibitors helenalin and carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (MG132) were purchased from CalBiochem Co., Taq DNA polymerase was from Viogene Co., Taipei, Taiwan. The sequence and source of TS, CX, LT5 and LT6 oligonucleotides were previously described [31]. The oligonucleotides WW144 (5′-TGG TAT CGT GGA AGG ACT CAT GAC-3′), WW145 (5′-ATG CCA GTG AGC TTC CCG TTC AGC-3′), cMyc-F (5′-AGAGTCTGGATCACCTTCTGCTGG-3′) and cMyc-R (5′-ACGGACAGGATGTATGCTGTGG-3′) were obtained from Protech Co., Taipei, Taiwan. Gel electrophoresis reagents were from Bio-Rad. Antibodies against NFκB p50, cMyc and Sp1 were purchased from Upstate, NY. Anti-human CD3 and anti-human CD28 were obtained from BioLegend. Antibodies against PKC α, β1, δ, ζ, and θ were from Santa Cruz Biotech. All other chemicals were from Sigma Co.

2.2. Plasmids

Plasmids that overexpress wild-type (WT) or constitutively active (CA) mutant form of PKC α, β1, δ, ζ [32], and θ [33] were kindly supplied by Drs. P. Parker and S. Shaw, respectively. The luciferase reporter plasmid pBTdel-408 [18] that contains the core promoter of hTERT was kindly supplied by Dr. I. Horikawa. The pSV-β-galactosidase plasmid was kindly provided by Dr. Y.S. Chang. The pGL3-basic and pGL3-control plasmids were obtained from Promega.

2.3. Cell culture

The peripheral blood mononuclear cells (PBMC) and lymphocytes (PBL) were isolated and cultured as previously described [30]. Nasopharyngeal carcinoma-derived cell line NPC-076 was cultured in DMEM as previously described [31].

2.4. Telomerase activity assay

A PCR-based telomeric repeat amplification protocol (TRAP) [34] was used for assaying telomerase activity. The preparation of cell extracts, the condition for PCR amplification and analysis of PCR products by electrophoresis on polyacrylamide gel were as described [30].

2.5. RT-PCR analysis

Total RNA was extracted by TRIzol isolation reagent following manufacturer's instruction. The primers used were: LT5 and LT6 for hTERT, cMyc-F and cMyc-R for cMyc, and WW144 and WW145 for GAPDH. The conditions for RT-PCR analysis were as described [30].

2.6. DNA transfection and reporter gene assay

The protocol for DNA transfection of unstimulated human T cells followed that suggested by Amaxa Biosystems. In brief, 5×10^6 freshly prepared PBMC were suspended in 0.1 ml of Human T Cell Nucleofector Solution (Amaxa Biosystems), mixed with 5 μg PKC-expressing plasmid DNA, 5 μg luciferase-reporter DNA and 1 μg pSV-β-galactosidase DNA, and transferred into an Amaxa certified cuvette. After inserting the cuvette into the cuvette holder of Nucleofector™, the cells were electroporated using program U-14. Electroporated T cells were quickly placed in RPMI 1640 medium containing 10% FBS, and cultured for 24 h. The cells were harvested and lysed in 50 μl of lysis buffer (Promega). Samples containing 50-μg protein were used to measure the luciferase activity in an illuminometer (Berthold autolumate model LB953). The β-galactosidase activity per transfection, determined with a spectrophotometer at OD_{420}, was used as the internal control. The pGL3-basic and pGL3-control plasmids were served as the negative and positive controls, respectively. Transfection of plasmid DNA into NPC-076 cells using LipofectAMINE™ 2000 followed the supplier's instructions.

2.7. Chromatin immunoprecipitation (ChIP) assay

The treated PBL was suspended in RPMI 1640 medium containing 1% formaldehyde (10^6 cells/ml), and incubated at 37 °C for 10 min. The crosslinking reaction was stopped by adding glycine to a final concentration of 0.125 M and the cells were collected by centrifugation. After washing twice with cold PBS containing 10 μM PMSF and protease inhibitor cocktail (Sigma), the cell pellets were stored at −70 °C. The preparation of cell lysates, condition for the sonication of DNA, and the isolation of antibody-bound chromatin followed that as described [35]. The sets of primers used for the detection of antibody-bound chromatin DNA in the hTERT promoter region include: (i) Region 0 (+47 to +356). F0: 5′-GCCACTACCGCGAGGTGCT-3′; R0: 5′-CACTCGGGCCACCAGCTCC-3′, (ii) Region 1 (−22 to −296). F1: 5′-TGCCCCTTCACCTTCCAG-3′; R1: 5′-CAGCGCTGCC-TGAAACTC-3′, (iii) Region 2 (−274 to −484). F2: 5′-ACA-TCATGGCCCCTCCCT-3′; R2: 5′-CTGGAAGGTGAAGGGGCA-3′, and (iv) Region 3 (−590 to −793). F3: 5′-GATTAACAGAT-TTGGGGT-3′; R3: 5′-AGGACGGGAGGCGCGTAGA-3′.

2.8. Western blot

Western blot analysis was performed as previously described [31].

3. Results and discussion

Expression of telomerase in activated T cells is known to require PKC-dependent induction of hTERT expression [28,30]. To determine the role of individual PKC isoform in the induction of hTERT, we employed a luciferase reporter to examine the effects of PKC overexpression on the promoter activity of hTERT in unstimulated T cells. Plasmid that overexpresses wild-type (WT) or constitutively-active mutant form (CA) of PKC, and a luciferase reporter plasmid were co-transfected into unstimulated T cells and the activation of hTERT promoter activity was analyzed after 24 h of culturing. As shown in Fig. 1A, overexpression of wild-type or constitutively-active mutant form of PKC α, β1, δ, and ζ did not activate hTERT promoter activity. In contrast, overexpression of PKC θ activated the hTERT promoter activity about 2–6 fold. Surprisingly, overexpression of wild-type PKC θ produced an even greater activation of the hTERT promoter than that produced by the CA-mutant. Analyses of PKC by Western blot, however, failed to detect any significant increase of PKC level in the T cells transfected with PKC plasmids (unpublished data). Presumably, the low transfection efficiency of the primary T cells masks the detection of ectopically expressed PKC over that of the endogenous ones. To remedy this technical problem, we examined the expression of PKC plasmids in a highly transfectable cells, NPC-076. As shown in Fig. 1B, the levels of PKC α, θ, and ζ, but not β1 or δ, were greatly increased in the cells transfected with PKC plasmids. Assuming that the levels of expression/stability are similar both in the transfected NPC-076 and T cells, these results indicate that only the overexpression of PKC θ could transactivate the hTERT expression. Although the underlying mechanism for the greater transactivation activity of the wild-type PKC θ is not known, it cannot be accounted simply by the higher levels of PKC expressed by the wild-type plasmid, since similar levels of ectopically expressed PKC were detected in the NPC-076 cells transfected with wild-type or mutant PKC θ plasmids.

Since PKC θ is known to play a central role in the signaling of T cell activation [25,26], we addressed which one of the PKC θ-dependent signaling pathways is involved in the induction of hTERT and telomerase expression. Although the down-stream signaling mediated by PKC θ is still not fully

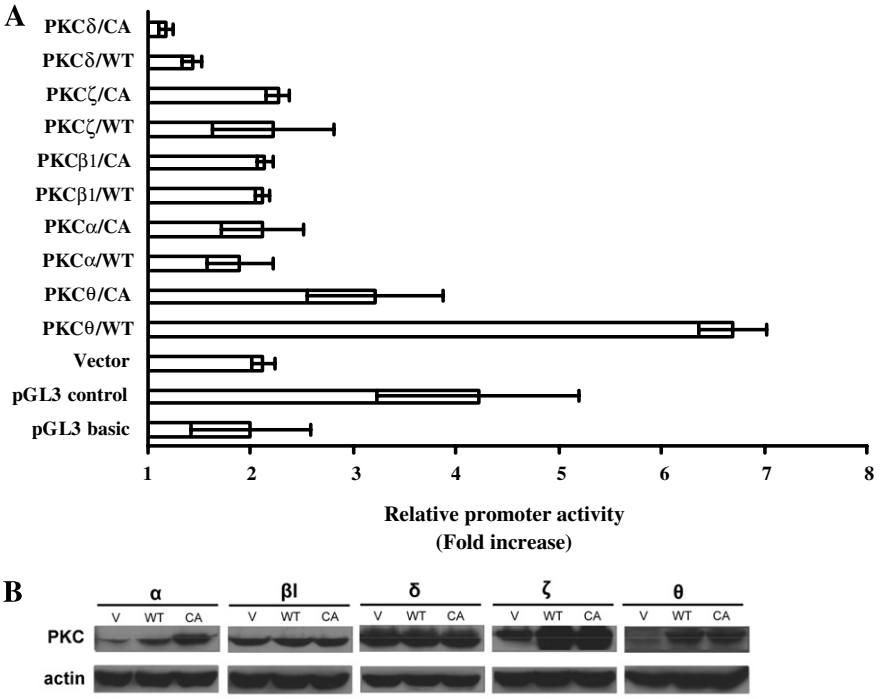

Fig. 1. Activation of the hTERT promoter by overexpression of individual PKC isoform. (A) Unstimulated T lymphocytes were co-transfected with a PKC-expressing plasmid and a luciferase reporter plasmid pBTdel-408, and assayed for luciferase activity as described in "Section 2". pGL3 control and pGL3 basic served as the positive and negative controls, respectively. Vector served as the control for comparison with the PKC-expressing plasmid. Values shown represent the results from two independent experiments with the indicated error bars. (B) NPC-076 cells were transfected with PKC plasmids and the expression of PKC isoform was monitored after 24 h by Western blot as described in "Section 2". The plasmids V, WT, and CA indicate the vector control, the wild-type PKC and the constitutively-active mutant PKC, respectively.

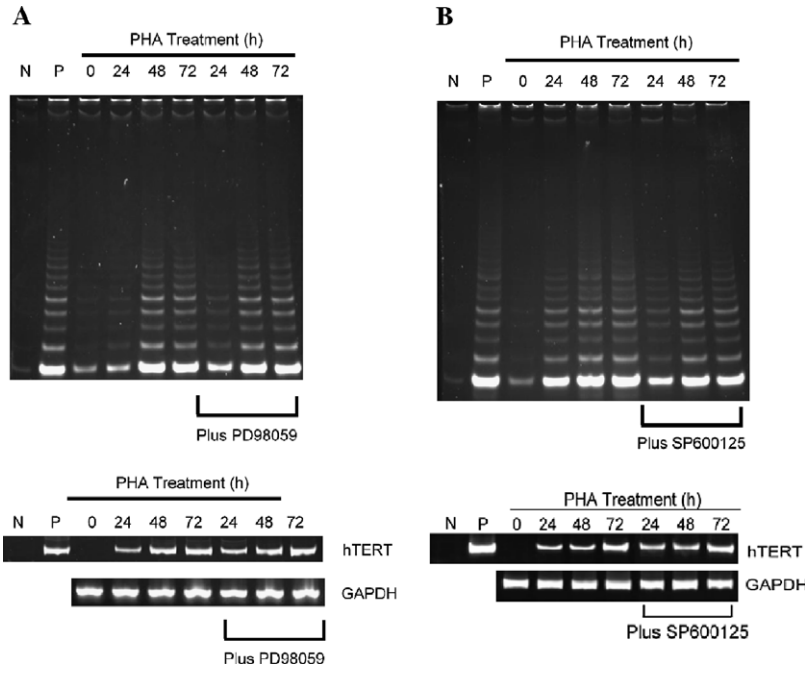

Fig. 2. Effect of PD98059 and SP600125 on the expression of hTERT and telomerase activity in PHA-treated PBL. PBL were treated with PHA in the presence or absence of 50 μM PD98059 (panel A), or 12.5 μM SP600125 (panel B) for the indicated times, and assayed for the expression of telomerase activity (top) and hTERT (bottom) as described in "Section 2". Lane P is a positive control using cell extract or RNA obtained from telomerase-positive HL-60 cells. Lane N is a negative control with no cell extract or RNA. The expression of GAPDH was included as a loading control in the RT-PCR analysis. Results shown are from one of three similar experiments.

established in activated T cells, activation of the MEK, JNK, and NFκB pathways has been shown to account for a number of major events observed in T cell activation [25–27]. Here, we employed inhibitors specific for MEK, JNK and NFκB to investigate the role of these pathways in the expression of hTERT and telomerase activity in activated T cells. As shown in Fig. 2A, the induction of hTERT expression and telomerase activity was essentially not affected by the presence of MEK inhibitor PD98059 in the phytohemagglutinin (PHA)-stimulated T cells. Similar results were also obtained with another MEK inhibitor U0126 (data not shown). Inhibition of JNK by SP600125 also did not inhibit the expression of hTERT and telomerase activity in the PHA-stimulated T cells (Fig. 2B), indicating that MEK and JNK are not involved in the induction of hTERT expression. In contrast, a complete inhibition of hTERT induction and telomerase activation was observed when the NFκB inhibitors MG132 or helenalin were added simultaneously with PHA (Fig. 3). A similar pattern of hTERT inhibition was also observed in the T cells activated by anti-CD3/CD28 (data not shown), indicating that the signaling through NFκB plays an important role in the expression of hTERT in the T cells activated with PHA or anti-CD3/CD28.

Our observation for a major role of NFκB in the transcriptional control of hTERT expression suggests that NFκB activates the hTERT promoter either directly or indirectly. Since putative NFκB binding sites are present in the hTERT promoter and a direct binding of NFκB to the mouse TERT promoter has been demonstrated [36], we investigated if the activation of hTERT expression may be mediated by a direct binding of NFκB to the hTERT promoter. We have employed chromatin immunoprecipitation assay to examine the hTERT promoter occupancy in vivo, and the results are shown in Fig. 4. NFκB-binding was detected in the Regions 0 and 1 of hTERT promoter in unstimulated lymphocytes but not in the activated cells. A weak NFκB-binding to Region 3 was detected in both unstimulated and activated T cells. Since the expression of hTERT is not detected in unstimulated T cells, the observed NFκB-binding in Regions 0 and 1 may in fact play a negative regulatory function for hTERT expression. The lack of increased NFκB-binding to the hTERT promoter in the activated T cells suggests that a direct NFκB-binding is unlikely to account for the transactivation of hTERT expression. Contrary to that observed for NFκB, an increased binding of cMyc and Sp1 to the hTERT promoter was detected in the PHA-treated cells (Fig. 4).

A major role of NFκB in the transcriptional regulation of hTERT has also been reported in HTLV-I-transformed T cells [37] and in estrogen-treated ovarian cancer cells [38]. In the case of estrogen-treated ovarian cancer cells, a PI3K/Akt/NFκB cascade is thought to regulate both the transcriptional expression and post-transcriptional phosphorylation of hTERT, although the molecular mechanism by which NFκB regulates the transcription of hTERT was not known. In the case of HTLV-I-transformed T cells, an increased binding of c-Myc and Sp1 but not NFκB is suggested to be involved in the NFκB-mediated activation of the hTERT promoter. Similar to the finding of Sinha-Datta et al. [37], our data of the hTERT promoter occupancy in vivo have revealed an increased binding of c-Myc and Sp1, but not NFκB, to the hTERT promoter in the activated T cells (Fig. 4), suggesting that NFκB-mediated activation of the hTERT may act via increased binding of c-Myc and Sp1.

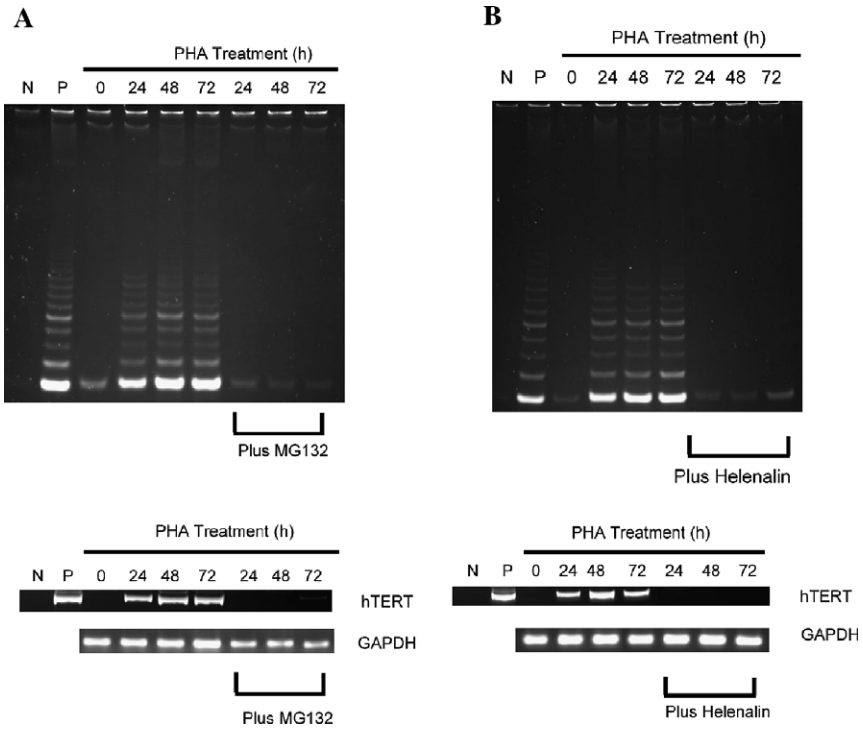

Fig. 3. Effect of NFκB inhibitors on the expression of hTERT and telomerase activity in PHA-treated PBL. PBL were treated with PHA in the presence or absence of 0.5 μM MG132 (panel A) or 1 μM helenalin (panel B), and assayed for the expression of telomerase activity (top) and hTERT (bottom) as described in "Section 2". Lane P is a positive control using cell extract or RNA obtained from telomerase-positive HL-60 cells. Lane N is a negative control with no cell extract or RNA. Results shown are from one of three similar experiments.

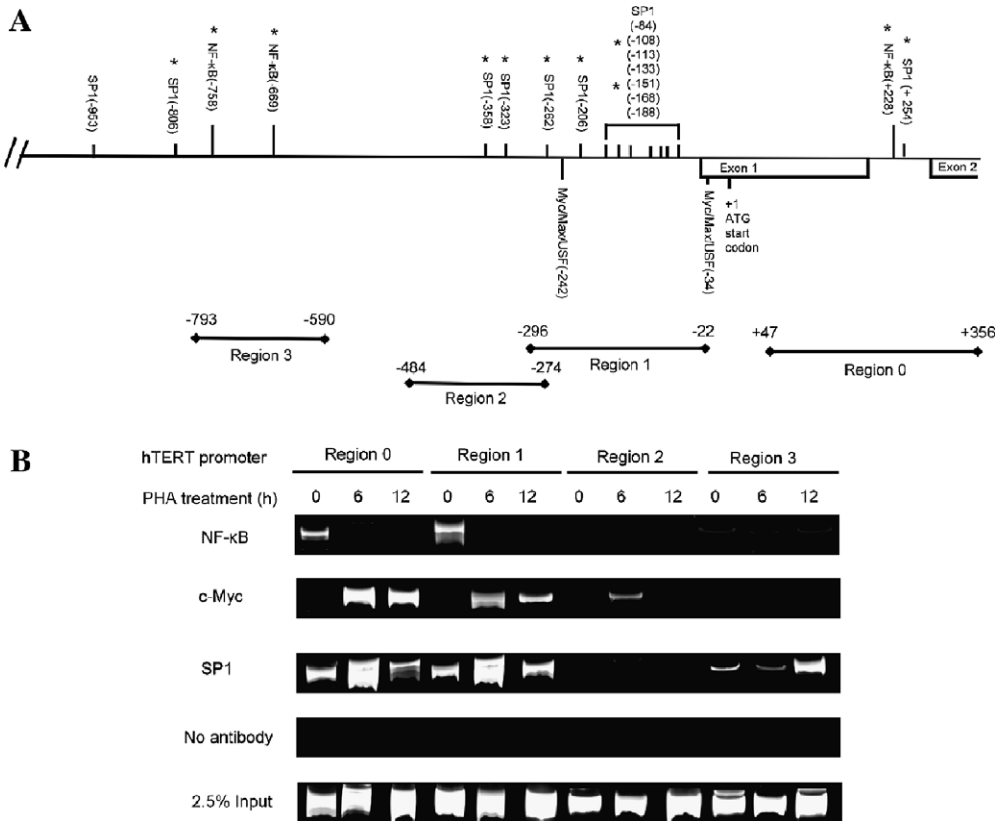

Fig. 4. hTERT promoter occupancy in vivo by chromatin immunoprecipitation assay (ChIP). (A) Schematic presentation of the hTERT promoter and the primer locations for Regions 0, 1, 2, and 3. The sites marked with star (*) are putative binding sites for Sp1 and NFκB. (B) hTERT promoter occupancy in vivo. PBL were treated with PHA for the indicated times and the chromatin bound by transcriptional factors Sp1, cMyc, and NFκB were analyzed by ChIP assay as described in "Section 2". The PCR products from 2.5% input samples and from DNA precipitated in the absence of antibody (No antibody) were included for comparison. Results shown are from one of two similar experiments.

Finally, since cMyc is one of activators known to transactivate hTERT promoter [39–41] and cMyc is known to be potently transactivated by NFκB [42], it raises the possibility that NFκB may activate the expression of cMyc which in turn transactivates the expression of hTERT. To test this postulate, we examined the effect of NFκB inhibitors on the expression of cMyc in activated T cells. As shown in Fig. 5, inhibition of NFκB completely abolished the induction of cMyc expression in the activated T cells, suggesting that the NFκB-mediated expression of cMyc is critical for the expression of hTERT in the activated T cells.

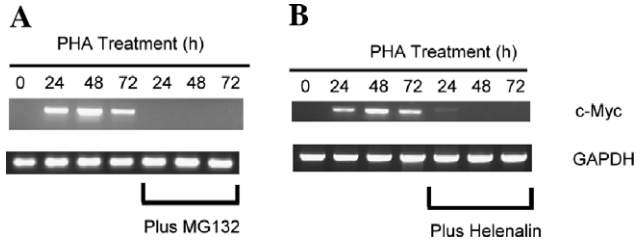

Fig. 5. Effect of NFκB inhibitors on the expression of *cMyc* in PHA-treated PBL. PBL were treated with PHA in the presence or absence of 0.5 μM MG132 (panel A) or 1 μM helenalin (panel B), and assayed for the expression of cMyc by RT-PCR as described in "Section 2". The expression of GAPDH served as a loading control. Results shown are from one of two similar experiments.

Taken together, these results establish that PKC θ is the PKC isoform that is involved in the transcriptional regulation of hTERT expression in T lymphocytes. Of the several PKC θ-mediated signaling pathways, we provide evidence that the expression of hTERT is mediated through NFκB but not through MEK or JNK pathways (Figs. 2 and 3). Although the mechanism by which NFκB mediates the activation of hTERT expression is not fully deduced at present, our results suggest that NFκB does not participate in the direct transactivation of hTERT promoter, but may potently activate cMyc expression which in turn transactivates the expression of hTERT in the activated T cells.

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References

[1] Cong, Y.S., Wright, W.E. and Shay, J.W. (2002) Human telomerase and its regulation. Microbiol. Mol. Biol. Rev. 66, 407–425.
[2] Blackburn, E.H. (1992) Telomerases. Annu. Rev. Biochem. 61, 113–129.
[3] Shay, J.W. and Roninson, I.B. (2004) Hallmarks of senescence in carcinogenesis and cancer therapy. Oncogene 23, 2919–2933.

[4] Wright, W.E. and Shay, J.W. (2001) Cellular senescence as a tumor-protection mechanism: the essential role of counting. Curr. Opin. Genet. Dev. 11, 98–103.
[5] Wright, W.E., Piatyszek, M.A., Rainey, W.E., Byrd, W. and Shay, J.W. (1996) Telomerase activity in human germline and embryonic tissues and cells. Dev. Genet. 18, 173–179.
[6] Weng, N.P., Levine, B.L., June, C.H. and Hodes, R.J. (1996) Regulated expression of telomerase activity in human T lymphocyte development and activation. J. Exp. Med. 183, 2471–2479.
[7] Masutomi, K., Possemato, R., Wong, J.M., Currier, J.L., Tothova, Z., Manola, J.B., Ganesan, S., Lansdorp, P.M., Collins, K. and Hahn, W.C. (2005) The telomerase reverse transcriptase regulates chromatin state and DNA damage responses. Proc. Natl. Acad. Sci. USA 102, 8222–8227.
[8] Masutomi, K., Yu, E.Y., Khurts, S., Ben-Porath, I., Currier, J.L., Metz, G.B., Brooks, M.W., Kaneko, S., Murakami, S., DeCaprio, J.A., Weinberg, R.A., Stewart, S.A. and Hahn, W.C. (2003) Telomerase maintains telomere structure in normal human cells. Cell 114, 241–253.
[9] Nakamura, T.M., Morin, G.B., Chapman, K.B., Weinrich, S.L., Andrews, W.H., Lingner, J., Harley, C.B. and Cech, T.R. (1997) Telomerase catalytic subunit homologs from fission yeast and human. Science 277, 955–959.
[10] Meyerson, M., Counter, C.M., Eaton, E.N., Ellisen, L.W., Steiner, P., Caddle, S.D., Ziaugra, L., Beijersbergen, R.L., Davidoff, M.J., Liu, Q., Bacchetti, S., Haber, D.A. and Weinberg, R.A. (1997) hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 90, 785–795.
[11] Harrington, L., McPhail, T., Mar, V., Zhou, W., Oulton, R., Bass, M.B., Arruda, I. and Robinson, M.O. (1997) A mammalian telomerase-associated protein. Science 275, 973–977.
[12] Nakayama, J., Saito, M., Nakamura, H., Matsuura, A. and Ishikawa, F. (1997) TLP1: a gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family. Cell 88, 875–884.
[13] Avilion, A.A., Piatyszek, M.A., Gupta, J., Shay, J.W., Bacchetti, S. and Greider, C.W. (1996) Human telomerase RNA and telomerase activity in immortal cell lines and tumor tissues. Cancer Res. 56, 645–650.
[14] Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S. and Wright, W.E. (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279, 349–352.
[15] Nakayama, J., Tahara, H., Tahara, E., Saito, M., Ito, K., Nakamura, H., Nakanishi, T., Tahara, E., Ide, T. and Ishikawa, F. (1998) Telomerase activation by hTRT in human normal fibroblasts and hepatocellular carcinomas. Nat. Genet. 18, 65–68.
[16] Vaziri, H. and Benchimol, S. (1998) Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. Curr. Biol. 8, 279–282.
[17] Counter, C.M., Meyerson, M., Eaton, E.N., Ellisen, L.W., Caddle, S.D., Haber, D.A. and Weinberg, R.A. (1998) Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase. Oncogene 16, 1217–1222.
[18] Horikawa, I., Cable, P.L., Afshari, C. and Barrett, J.C. (1999) Cloning and characterization of the promoter region of human telomerase reverse transcriptase gene. Cancer Res. 59, 826–830.
[19] Takakura, M., Kyo, S., Kanaya, T., Hirano, H., Takeda, J., Yutsudo, M. and Inoue, M. (1999) Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. Cancer Res. 59, 551–557.
[20] Cong, Y.S., Wen, J. and Bacchetti, S. (1999) The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. Hum. Mol. Genet. 8, 137–142.
[21] Ducrest, A.L., Szutorisz, H., Lingner, J. and Nabholz, M. (2002) Regulation of the human telomerase reverse transcriptase gene. Oncogene 21, 541–552.
[22] Lin, S.Y. and Elledge, S.J. (2003) Multiple tumor suppressor pathways negatively regulate telomerase. Cell 113, 881–889.
[23] Poole, J.C., Andrews, L.G. and Tollefsbol, T.O. (2001) Activity, function, and gene regulation of the catalytic subunit of telomerase (hTERT). Gene 269, 1–12.
[24] Liu, K., Schoonmaker, M.M., Levine, B.L., June, C.H., Hodes, R.J. and Weng, N.P. (1999) Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. Proc. Natl. Acad. Sci. USA 96, 5147–5152.
[25] Isakov, N. and Altman, A. (2002) Protein kinase C(theta) in T cell activation. Annu. Rev. Immunol. 20, 761–794.
[26] Meller, N., Altman, A. and Isakov, N. (1998) New perspectives on PKCtheta, a member of the novel subfamily of protein kinase C. Stem Cells 16, 178–192.
[27] Wilkinson, S.E. and Nixon, J.S. (1998) T-cell signal transduction and the role of protein kinase C. Cell Mol. Life Sci. 54, 1122–1144.
[28] Bodnar, A.G., Kim, N.W., Effros, R.B. and Chiu, C.P. (1996) Mechanism of telomerase induction during T cell activation. Exp. Cell Res. 228, 58–64.
[29] Igarashi, H. and Sakaguchi, N. (1996) Telomerase activity is induced by the stimulation to antigen receptor in human peripheral lymphocytes. Biochem. Biophys. Res. Commun. 219, 649–655.
[30] Sheng, W.Y., Chien, Y.L. and Wang, T.C. (2003) The dual role of protein kinase C in the regulation of telomerase activity in human lymphocytes. FEBS Lett. 540, 91–95.
[31] Yu, C.C., Lo, S.C. and Wang, T.C. (2001) Telomerase is regulated by protein kinase C-zeta in human nasopharyngeal cancer cells. Biochem. J. 355, 459–464.
[32] Wooten, M.W., Seibenhener, M.L., Soh, Y., Ewald, S.J., White, K.R., Lloyd, E.D., Olivier, A. and Parker, P.J. (1992) Characterization and differential expression of protein kinase C isoforms in PC12 cells. Differentiation parallels an increase in PKC beta II. FEBS Lett. 298, 74–78.
[33] Liu, Y., Graham, C., Li, A., Fisher, R.J. and Shaw, S. (2002) Phosphorylation of the protein kinase C-theta activation loop and hydrophobic motif regulates its kinase activity, but only activation loop phosphorylation is critical to in vivo nuclear-factorκB induction. Biochem. J. 361, 255–265.
[34] Kim, N.W., Piatyszek, M.A., Prowse, K.R., Harley, C.B., West, M.D., Ho, P.L., Coviello, G.M., Wright, W.E., Weinrich, S.L. and Shay, J.W. (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266, 2011–2015.
[35] Weinmann, A.S. and Farnham, P.J. (2002) Identification of unknown target genes of human transcription factors using chromatin immunoprecipitation. Methods 26, 37–47.
[36] Yin, L., Hubbard, A.K. and Giardina, C. (2000) NF-κB regulates transcription of the mouse telomerase catalytic subunit. J. Biol. Chem. 275, 36671–36675.
[37] Sinha-Datta, U., Horikawa, I., Michishita, E., Datta, A., Sigler-Nicot, J.C., Brown, M., Kazanji, M., Barrett, J.C. and Nicot, C. (2004) Transcriptional activation of hTERT through the NF-κB pathway in HTLV-I-transformed cells. Blood 104, 2523–2531.
[38] Kimura, A., Ohmichi, M., Kawagoe, J., Kyo, S., Mabuchi, S., Takahashi, T., Ohshima, C., Arimoto-Ishida, E., Nishio, Y., Inoue, M., Kurachi, H., Tasaka, K. and Murata, Y. (2004) Induction of hTERT expression and phosphorylation by estrogen via Akt cascade in human ovarian cancer cell lines. Oncogene 23, 4505–4515.
[39] Greenberg, R.A., O'Hagan, R.C., Deng, H., Xiao, Q., Hann, S.R., Adams, R.R., Lichtsteiner, S., Chin, L., Morin, G.B. and DePinho, R.A. (1999) Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. Oncogene 18, 1219–1226.
[40] Wang, J., Xie, L.Y., Allan, S., Beach, D. and Hannon, G.J. (1998) Myc activates telomerase. Genes Dev. 12, 1769–1774.
[41] Wu, K.J., Grandori, C., Amacker, M., Simon-Vermot, N., Polack, A., Lingner, J. and Dalla-Favera, R. (1999) Direct activation of TERT transcription by c-MYC. Nat. Genet. 21, 220–224.
[42] La Rosa, F.A., Pierce, J.W. and Sonenshein, G.E. (1994) Differential regulation of the c-myc oncogene promoter by the NF-κB rel family of transcription factors. Mol. Cell Biol. 14, 1039–1044.